DI-n-OCTYL PHTHALATE (DnOP)

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Di-n-Octyl Phthalate--Contents

1.0 EXPOSURE

1.1 Chemistry

Di-n-octyl phthalate (DnOP) (CAS number 117-84-0) is produced by reacting phthalic anhydride and n-octanol in the presence of an acid catalyst (*2*).

Synonyms: 1,2-benzenedicarboxylic acid, dioctyl ester; phthalic acid, dioctyl ester; n-dioctyl phthalate; n-octyl phthalate; dioctyl o-benzenedicarboxylate; bis(n-octyl) phthalate.

DnOP is a significant component (20%) of C6–10 phthalate mixtures (*2*).

Table 1: Physicochemical Properties of DnOP

Property	Value
Chemical Formula	$C_{24}H_{38}0_4$
Molecular Weight	390.54
Vapor Pressure	1.0×10^{-7} mmHg at 25 ^{o}C
Melting Point	-25 ^{o}C
Boiling Point	390 ^{o}C
Specific Gravity	0.978
Solubility in Water	essentially insoluable (0.5 μg/L)
Log K_{ow}	8.06

(*10*)

1.2 Exposure

There is sometimes confusion reporting data for DnOP because DEHP is often referred to in the literature as dioctyl phthalate (DOP). Unless otherwise stated, the information discussed in this exposure section refers specifically to DnOP to the best of CERHR's knowledge.

There are no known commercial uses for pure DnOP. However, DnOP constitutes approximately 20% of C6–10 phthalate substance. Commercial production of 50 million pounds of C6-10 phthalate in the United States in 1994 (*1*) equates to 10 million pounds of DnOP. C6-10 phthalate substance is used in PVC utilized in the manufacture of flooring and carpet tile, canvas tarps, swimming pool liners, notebook covers, traffic cones, toys, vinyl gloves, garden hoses, weather stripping, flea collars, and shoes (*2*). DnOP-containing phthalate substances are also used in PVC intended for food applications such as seam cements, bottle cap liners, and conveyor belts.

Release of DnOP to the environment can occur during the production of C6-10 phthalates and during the incorporation of the phthalates into plastic resins. Because phthalates are not bound to plastics, DnOP can be released during the use or disposal of the product. Phthalates released to the environment can be deposited on or taken up by crops intended for human or livestock consumption, and thus, may enter the human food supply.

General Population Exposure

The general population is exposed to phthalates primarily through the oral and dermal routes. Based on data for other phthalates, the most likely source of DnOP exposure to humans is dietary intake. DnOP may be found in food as a result of environmental uptake during cultivation or as a result of migration from processing equipment or packaging materials. DnOP is approved for use as an indirect food additive in sealants used for food packaging (*3*). In a survey of packaged fatty foods purchased from grocery stores in the UK, the total concentration of dioctyl phthalate (DOP, isomer not specified) *excluding* DEHP was 2.3 mg/kg in milk (*4*). A paper published in 1995 reported the detection of DnOP in two samples of vodka; concentrations of 57 ppb in a 100 proof sample and 131 ppb in an 80 proof vodka (*5*).

DOP (isomer not specified) excluding DEHP was detected in 8 of 12 infant formulas from the UK at concentrations ranging from 0.21–1.42 mg/kg (*6*). Using manufacturer recommendations for feeding rates and by assuming that formula was the only nutritional source for infants, exposures to DOP isomers other than DEHP were estimated at <0.1–43 μg/kg bw/day at birth and <0.1–24 μg/kg bw/day at 6 months of age by MAFF (*6*). In a follow-up survey DOPs were not specifically targeted but there was no evidence of their presence in 39 sample of infant formulas examined (*7*).

There appears to be little or no use of DnOP-containing compounds in toys. According to the CMA (*2*), DnOP was only detected in some teethers that were tested for phthalate ester migration by the Danish Ministry of Environment and Energy. No other studies have reported the detection of DnOP in toys.

Exposure to DnOP through air is also possible but expected to be minimal. Reported concentrations of DnOP in ambient air range from 0.06 to 0.94 ng/m^3. The highest reported concentration resulted in a calculated inhaled dose of 0.29 ng/kg bw/day for an adult (*8*). Reported concentrations in river water have ranged from 0.024 to 1 ppb. EPA estimates that drinking water influents are less than 0.5 ppb (*8*). These levels are several orders of magnitude less than levels found in food.

The available data do not allow the confident estimation of DnOP exposures to the general population. However, a comparison of production volumes for DnOP-containing compounds versus those that contain DEHP suggests that human exposure to DnOP is well below the exposure estimate for DEHP of 3–30 μg/kg bw/day (*9*).

Medical Exposure

There are no known uses of DnOP-containing compounds in medical devices.

Occupational Exposure

Workers may be exposed to DnOP primarily through inhalation and dermal contact. Phthalates are manufactured within closed systems, but exposure to workers can occur during filtering or loading/unloading of tank cars (*2*). Higher exposures to phthalates can occur during the production of flexible PVC because the processes are open and run at higher temperatures. According to the CMA (*2*), phthalate levels in air are generally less than 1 mg/m^3 and 2 mg/m^3 during the production of phthalates and flexible PVC, respectively. Exposure levels were estimated by the CMA (*2*) using assumptions of a 10 m^3/day inhalation rate and a 70 kg body weight. The resulting exposure estimates were 143 μg/kg bw/day and 286 μg/kg bw/day for workers employed in phthalate and flexible PVC manufacturing operations, respectively. If the total number of days worked per year is assumed to be 220 days, the exposure estimates convert to 86 and 172 μg/kg bw/day.

Summary

See Section 5.1.1 for summary of exposure data.

2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS

2.1 General Toxicity

2.1.1 Human Data

There were no data on human toxicity available.

2.1.2 Experimental Animal Data

Acute Toxicity: The LD_{50} values for mice and rats are given as 13 g/kg and 53.7 g/kg, respectively. Dermal LD_{50} values have been determined at 75 mL/kg for guinea pigs (*2*).

Repeat-dose Toxicity: Three studies in rodents were available for review (*11-14*).

Systemic effects following DnOP treatment for either 3, 10, or 21 days were examined in 4-week-old Wistar rats. These effects were compared to effects produced by other groups of rats being fed diet containing 20,000 ppm of di-n-hexyl phthalate (DnHP), another straight phthalate, or di(2-ethylhexyl) phthalate (DEHP), a branched-chained phthalate (*13*). A group of 12 male rats was a fed a diet containing 20,000 ppm DnOP and a control group of 18 rats was fed the basal diet. Using actual food intake levels and rat body weights on the day of sacrifice, a DnOP dose of 1,821 mg/kg bw/day was calculated. Four treated and 6 control rats were killed and necropsied after 3, 10, and 21 days of treatment. Liver histopathology, enzyme activity, and peroxisome proliferation were examined. Levels of thyroid hormones in serum and thyroid histopathology were also examined (*11*).

DnOP treatment had no effect on testes weight or the gross appearance of testes, kidney, or pancreas (*13*). However, liver weight was significantly increased at 10 and 21 days of DnOP treatment with liver histology and chemistry changes seen at all three assessment times. After 3 days of exposure, centrilobular necrosis and glycogen loss were observed. At 10 days, centrilobular fatty accumulation was seen and this effect became more pronounced with increasing treatment duration. Electron microscopy (EM) showed effects on the smooth endoplasmic reticuli (proliferation and dilation) and microvilli shortening in the bile canaliculi at 3 days. At 10 days, EM also showed lipid droplets in the hepatocytes and a small increase in lysosomes and peroxisomes at 3 and 21 days respectively. Biochemical evidence for peroxisomal proliferation was seen with significant increases in cyanide insensitive palmitoyl CoA oxidase at 10 and 21 days of treatment. Total catalase activity was unchanged; however, in the particulate sub-fraction it was significantly increased at 10 and 21 days of treatment. Other liver enzymes that were changed included significant decreases in 5'-nucleotidase, succinate dehydrogenase, and glucose-6-phosphate at 21 days of treatment. There was a significant, DnOP-treatment related decrease in serum thyroxine (T4); serum triiodothyronine levels (T3) were not significantly affected and microscopic changes were suggestive of thyroid hyperactivity. These changes included increased lysosomal numbers and size, enlarged Golgi apparatus, and mitochondrial damage.

When compared to the other two co-tested phthalates, DnOP induced effects on hepatic lipid accumulation and peroxisomal proliferation that were similar to DnHP but dissimilar to DEHP. DEHP caused greater increases in liver weight and greater increases in mitotic activity. Less fat accumulation was seen with DEHP treatment which, when it occurred, was seen in the midzone and periportal zones rather than centrilobular regions. Biochemical evidence for peroxisomal proliferation (cyanide insensitive palmitoyl CoA oxidation) occurred earlier with DEHP (after 3 days of treatment) and was approximately 7-fold higher than the levels observed with DnOP. DnOP values were twice those observed in the control rats.

An effect level of 1,821 mg/kg bw/day was observed in this study after 3 days of treatment. Although DEHP was a stronger inducer of peroxisome proliferation, rats exposed to DnOP did show evidence for proliferation after longer treatment. Additional liver effects suggestive of other types of liver damage were seen with DnOP.

Liver metabolism and the biochemical changes associated with peroxisome proliferation were also studied by Lake et al. (*12*) who treated rats with 1,000 mg/kg DnOP for 14 days. DnOP produced a marginal increase in liver weight compared with DEHP. There were no increases in peroxisomal enzyme activities at 1,000 or 2,000 mg/kg.

Systemic effects were studied (*14*) in groups of young (~4–6 weeks old) Sprague-Dawley rats. Groups of (10/sex) were fed DnOP at dietary concentrations of 0, 5, 50, 500, or 5,000 ppm (males: 0, 0.4, 3.5, 36.8, or 350 mg/kg bw/day; females: 0, 0.4, 4.1, 40.8, or 403 mg/kg bw/day) for 13 weeks (Table WEB1). Negative controls (10/sex) were fed basal diet and positive controls (10/sex) were fed 5,000 ppm DEHP (males: 345 mg/kg bw/day; females: 411 mg/kg bw/day). Rats were observed daily, and body weights and food intake were measured weekly. At the end of the exposure period rats were killed and necropsied. Parameters evaluated included histopathology (reproductive organs preserved in Zenker's solution), hematology, blood chemistry, liver enzyme activity, peroxisome proliferation, and DnOP levels in tissues.

DnOP exposure did not affect organ or body weight at any dose concentration. No hematological effects or testicular changes were observed. At the dose of 4.1 mg/kg bw/day (F) rats experienced significant increases in phosphate. At 36.8 (M) 40.8 (F) mg/kg bw/day, no effects were observed and this level was designated by the authors as a NOAEL. At the highest DnOP exposure tested, 350.1 (M) and 402.9 (F) mg/kg bw/day, liver and thyroid effects were observed. Authors reported dose-related hepatic effects including anisokaryosis, nuclear hyperchromicity, vesiculation, cytoplasmic vacuolation, nuclear endothelial prominence, and accentuation of zonation. Increases in hepatic ethoxyresorufin-o-deethylase activity were also seen in this high-dose group. Thyroid effects, observed at the highest dose of DnOP tested, included a decrease in follicle size and colloid density. Serum T3 or T4 analyses for thyroid function were not performed. Plasma calcium levels were significantly increased in male rats at the high dose only.

The DEHP-positive control group of rats, exposed to 345 (M) and 411(F) mg/kg bw/day, had effects in the liver and thyroid with respect to severity of lesions and biochemical changes that were similar to those observed in the high-dose DnOP group. However, DEHP also induced peroxisomal proliferation, seminiferous tube atrophy, Sertoli cell vacuolation, and decreased sperm levels. Also, numerous additional biochemical changes were observed with the DEHP-exposed rats, such as increases in plasma levels of albumin and inorganic phosphate, total protein (F), and hepatic aminopyrine-N-demethylase and analine hydroxylase. DEHP also produced significant hematological effects such as increased platelet counts, increased WBC (F), and decreased mean corpuscular volume(F) and decreased hemoglobin (F).

Poon et al. (*14*) also evaluated tissue levels of DnOP and DEHP in liver and fat. Levels of DnOP in liver were 5 ppm in males of the 350 mg DnOP/kg bw/day group and 4, 5, and 4 ppm in females of the 4.1, 40.8, and 403 mg DnOP/kg bw/day groups, respectively. DnOP levels in fat tissue were 4, 7, and 15 ppm for

males in the 3.5, 36.8, and 350 mg DnOP/kg bw/day groups, respectively, and 7 and 25 ppm for females in the 0.4 and 403 mg/kg bw/day group, respectively. DEHP levels in the positive controls included hepatic levels of 3 ppm in males and females and fat levels of 23 and 31 ppm in males and females, respectively. The authors stated that the findings suggest that both DnOP and DEHP are rapidly metabolized and excreted and that their distribution in body tissues is determined by the lipophilicity of the compounds.

Summary

See Section 5.1.2 for summary of general toxicity studies.

2.2 Toxicokinetics

Absorption

Few data are available describing the toxicokinetics of DnOP. Albro and Moore (*15*) dosed male CD rats by gavage with 0.2 mL DnOP and collected urine for analysis of metabolites. They recovered 31% of the dose in the urine by 48 hours. The monoester and some free phthalic acid were detected, but no parent DnOP was observed. Blood levels of the monoester, mono-octylphthalate, were measured in rats following administration of 2,000 mg/kg of DnOP by gavage (*16*). The biological half-life in the blood was 3.3 hours with an area under the curve (AUC) of 1,066 µg·h/mL. Peak blood levels were observed at 3 hours following administration.

Biotransformation

Humans In a study comparing the relative rates of monohydrolysis of DnOP by rat, baboon, and human gut preparations, Lake et al. (*17*) demonstrated that these species possess similar intrinsic esterase activity. Rates observed in human intestinal preparations were similar enough to the other species to conclude that human intestinal metabolism of DnOP would be expected to result in absorption of the monoester similar to what occurs in rats.

Rodents Six dialkyl phthalates, including DnOP, were found to be metabolized to their monoesters by enzymes present in gut tissues. It is generally accepted that orally-ingested phthalate diesters are primarily hydrolyzed by esterases in the wall of the small intestine, not by gut flora, and absorbed almost entirely as the corresponding monoester (*18*).

Distribution

Following an oral dose of 2,000 mg/kg DnOP, mono-octylphthalate was found in blood and testes in 3–6 hours. (*19*).

Excretion

Following a gavage dose of 559 mg/kg for 2 days, metabolites accounting for 31% of the administered dose were found in urine. The major metabolites found in urine of rats were derived from the monoester. (*15*).

Side-Chain Associated Toxicokinetics

Octanol is oxidized to the fatty acid and metabolized by the fatty acid oxidation pathway.

Summary

See Section 5.1.2 for a summary of toxicokinetics data.

2.3 Genetic Toxicity

Mixtures containing DnOP have not shown conclusive evidence of mutagenicity. Barber et al. (*20*) tested 6,10-phthalate, which contains approximately 20% DnOP, in the mouse lymphoma mutation and Balb/3T3 cell transformation assays. Negative results were obtained in the cell transformation assay, but results of the mouse lymphoma mutation assay were considered equivocal due to the non-dose related increase in mutation frequency in non-activated cells.

Di(n-octyl, n-decyl) phthalate, which contains DnOP as a component, has been reported to be negative in the Ames test (*21*) and the Chinese hamster ovary/HPRT locus assay (*22*).

Summary

See Section 5.1.2 for summary of genetic toxicity data.

3.0 DEVELOPMENTAL TOXICITY

3.1 Human Data

There were no data located on the developmental toxicity of DnOP in humans.

3.2 Experimental Animal Toxicity

Two studies were found, one in rats by intraperitoneal injection and one in mice by gavage. A third study examined effects of metabolite exposure in rats.

Singh et al. **(*23*)** administered DnOP at 0, 5, or 10 mL/kg (equivalent to 0, 4,890, and 9,780 mg/kg based on the density of DnOP of 0.978 g/mL) by intraperitoneal injection to Sprague Dawley rats. The rats, 5 per group, were dosed on gd 5, 10, and 15. The control group was untreated or dosed with distilled water, normal saline, or cottonseed oil. Dams and fetuses were evaluated on gd 20. Information on maternal toxicity was not reported. Fetal body weight was reduced at both doses, and incidences of gross malformations were increased in a dose-related manner (0–2% in controls, 16% at 4,890 mg/kg, and 27% at 9,780 mg/kg). The abnormalities were predominantly missing tail, anophthalmia, twisted hind legs, and hematomas.

Hardin et al. **(*24*)** evaluated DnOP in the Chernoff-Kavlock assay in CD-1 mice. The mice, 40/group, were dosed by gavage, with 9,780 mg/kg bw/day (undiluted chemical, 10 mL/kg/day) or corn oil on gd 6–13. Dams were allowed to deliver their litters; dams and pups were terminated on pnd 3. No dams died, 39/40 had live litters, and maternal weight change was similar to controls. Litter size on pnd 0 was significantly reduced (10.2) versus the control value (11.5). Birth weight was normal as was pup survival to pnd 4. However, weight gain, on pnd 1–3, was significantly reduced (0.6 g) versus the control value (1.0 g).

There was no effect on ability to produce litters, litter size, sex ratio, or pup weight or viability in F_1 and F_2 litters in a continuous breeding study in CD-1 mice. Mice were exposed to 0, 1.25, 2.5, or 5% DnOP (0, 1,800, 3,600, or 7,500 mg/kg bw/day) (*25, 26*). Complete details of this study are included in Section 4.

Hellwig and Jackh (*27*) investigated the prenatal toxicity of n-octanol, a primary metabolite of DnOP, when administered by gavage to pregnant Wistar rats on days 6–15 of gestation. There were 6 groups studied (8–10 female/group) a distilled water control, an emulsifier control, and DnOP doses of 1, 5, 7.5, and 10 mmol/kg equal to 130, 650, 945, and 1,300 mg/kg bw/day DnOP, respectively. Dose-related symptoms of clinical intoxication of the nervous system were observed with maternal death seen in the three highest dose levels. A slight decrease in food consumption and body weight gain was also recorded at these doses. However, no effects on fetal weight, viability, or developmental toxicity were observed. The incidence of malformations was similar to that of controls.

3.3 Summary

See Section 5.1.3 for summary of developmental toxicity studies.

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

There were no data located that studied the effect of DnOP on human reproduction..

4.2 Experimental Animal Toxicity

One reproductive toxicity study was found for DnOP, that was reported by Heindel et al (*26*) (also (*25*) (Table WEB 2). In this Continuous Breeding study, CD-1 (Swiss) mice, 20 pairs/dose level, 40 in controls) were fed DnOP in the diet at 0, 1.25, 2.5, or 5% (w/w). Body weights and food consumption were monitored, and these concentrations gave calculated daily DnOP consumption of 1,800, 3,600, and 7,500 mg/kg bw/day. Following a week of pre-mating exposure, mice were housed as breeding pairs for 14 weeks. Litters born during the 14-week period were evaluated and removed so that the adult pair could continue breeding. Reproductive function was measured by determining the fertility index; litters/pair; live pups per litter; and pup sex, body weight, and gross external malformations.

There was no effect on ability to produce litters, litter size, sex ratio, or pup weight or viability over 5 successive litters. For this protocol, when no effect on fertility was seen, the last litter from both the high dose and control group was reared, and used to evaluate fertility and toxicity of the F_1 generation. In addition to the reproductive parameters evaluated in the F_0 mice, sperm morphology, estrous cycles, and selected organ weights were evaluated in the F_1 mice. The F_0 mice were discarded without necropsy after weaning the last litter. The F_1 animals were mated within dose groups at sexual maturity. DnOP had no effect on indices of fertility, litter size, or pup weight or viability. The control and high-dose F_1 adults were killed and necropsied after delivery of a single litter. DnOP at 7,500 mg/kg bw/day in the diet had no effect on male body weight, but increased absolute and relative liver weight and decreased relative seminal vesicles weight. Sperm indices were unchanged. In females, body weight was unchanged, while relative liver and kidney weights were increased; estrous cycle was unchanged by 7,500 mg/kg bw/day DnOP consumption.

As discussed in Section 2.2, Poon et al (*14*) (Table WEB 1) reported a subchronic-type study of DnOP. Pubertal SD rats were exposed to DnOP in diet at doses as high as 5,000 ppm (350 mg/kg bw/day) for 23 weeks when the rats were killed and necropsied. Testes were weighed and fixed in Zenker's solution; no sperm measures were taken. Terminal weights of whole body and testis were unaffected by DnOP consumption. Testis histology was normal.

No reproductive effects were seen, so no LOAEL can be determined. The reproductive NOAEL in this study is 5,000 ppm, ~350 mg/kg bw/day, based on lack of changes in testis weight and histology as observed by light microscopy.

Confidence in the quality of the study is moderate-to-high. Confidence that this study found the true NOAEL is moderate-to-low, because the right endpoints were not examined in gestationally-exposed animals.

Mode of Action

Following exposure to a variety of phthalate monoesters over a range of doses, germ cell detachment was examined in *in vitro* co-cultures of Sertoli-germ cells isolated from pubertal rats. Results indicate that the n-octyl monoester is ~100-fold less potent than the 2-ethylhexyl monoester in producing this effect (*28*). These co-culture *in vitro* studies suggest that DnOP produces a similar effect to other phthalates in this model system, albeit at concentrations two orders of magnitude greater. There are no *in vivo* data to suggest effects on either germ cells or Sertoli cells due to DnOP exposure.

Hormonal Activity

The estrogenic activity of DnOP has been examined using a battery of short-term *in vitro* and *in vivo* assays. DnOP did not compete with tritiated estradiol for binding to the rat uterine cytosolic estrogen receptor (*29*). DnOP did not induce any activity in *in vitro* gene expression assays systems and did not induce reporter gene activity in transiently transfected MCF-7 cells (*29*). DnOP, in contrast to the positive control estradiol, did not significantly induce a vaginal cornification response at any of the concentrations tested (20, 200, and 2,000 mg/kg) over the course of a 5-day experiment using immature and adult ovariectomized Sprague Dawley rats (*29*). The effect of subcutaneous injection of 10^{-4} mol of DBP, BBP, and DnOP on uterine vascular permeability following a 4-hour incubation was examined in mature ovariectomized Swiss albino mice (*30*). No significant effect on uterine vascular permeability was reported.

4.3 Summary

See Section 5.1.4 for a summary of reproductive toxicity effects.

5.0 DATA SUMMARY & INTEGRATION

5.1 Summary

5.1.1 Human Exposure

There are no known commercial uses for pure DnOP. However, DnOP constitutes approximately 20% of the commercial mixture C6,10-phthalate. This commercial mixture has a variety of home and consumer product uses as listed in Section 1.

Dietary. In a survey of infant formulas from the UK, the level of dioctyl phthalates (DOP) other than DEHP, ranged from 0.21–1.42 mg/kg (*6*). In a subsequent survey conducted in 1998, DOP isomers were not targeted but there was no evidence that they were present in 39 samples of infant formula tested (*7*). A paper published in 1995 has reported the detection of DnOP in two samples of vodka at concentrations of 57 and 131 ppb (*5*). Dioctyl phthalate is approved for use as an indirect food additive in sealants used for food packaging (*3*).

Exposure Estimates: Based on levels of DOP isomers (excluding DEHP) detected in baby formula, infant exposures to DOP isomers other than DEHP were estimated at <0.1–43 μg/kg bw/day at birth and <0.1–24 μg/kg/day at 6 months of age by MAFF (*6*). However there was no evidence that DOP isomers were present in infant formulas in a survey conducted 2 years later by MAFF (*7*).

Based on production volumes of DnOP-containing compounds versus those containing DEHP, human exposure in DnOP is likely lower than exposure to DEHP, which was estimated at 3–30 μg/kg bw/day (*9*). In occupational settings, exposure is thought to be highest in workers of flexible PVC manufacturing facilities. Based on general levels of phthalates reported, the CMA (*2*) estimated an exposure level of 286 μg/kg bw/day with an average yearly exposure of 172 μg/kg bw/day.

Utility of the Data to the CERHR Evaluation. There is very limited information on exposure and exposure pathways to DnOP in humans. DnOP is not known to be produced directly for commercial use but is a component (20%) in commercial 6–10 phthalate substances. 6–10 phthalates are used in a variety of consumer products. Dioctyl phthalates have been detected in foods and infant formulas in the United Kingdom.

5.1.2 General Biological and Toxicological Data

Data presented in this section are derived from experimental animal and laboratory studies. Human data were not found.

General toxicity. A 3-week dietary study (*11, 13*) and a 90-day dietary study (*14*) in rats have been conducted. Liver effects were noted when rats were fed 1,821 mg/kg bw/day for 3, 10, and 21 days, or 350 mg/kg bw/day for 90 days. Thyroid effects also were noted in rats fed 350 mg/kg/d for 90 days and 1,821 mg/kg bw/day for 21 days. No effects were observed in the testes in either study. The sub-chronic dietary NOAEL in rats is 36 (M)–40 (F) mg/kg bw.

Toxicokinetics. DnOP is metabolized and rapidly absorbed in the gut as the monoester and primarily excreted in the urine of rats. (*15, 16, 18*). The major metabolites found in urine of rats were derived from the monoester (*15*).

Genetic toxicity. DnOP has not been tested for genetic toxicity. Mixtures containing DnOP have not shown conclusive evidence of mutagenicity. Barber et al. (*20*) tested 6,10-phthalate in the mouse lymphoma mutation and Balb/3T3 cell transformation assays. Negative results were obtained in the cell transformation assay, but results of the mouse lymphoma mutation assay were considered equivocal due to the non-dose related increase in mutation frequency in non-activated cells. Di(n-octyl, n-decyl) phthalate, which contains DnOP as a component, has been reported to be negative in the Ames test (Jones and Churchill, 1991) and the Chinese hamster ovary/HPRT locus assay (*22*).

Utility of the Data to the CERHR Evaluation. The data is adequate for the examination of systemic effects. The data set consisted of one quality study that examined systemic effects in groups of rats exposed for 90 days to multiple doses of DnOP by the oral route, a route that is relevant to human exposure. Levels of DnOP in the diet were verified. The evaluation included a histological examination of various organs, including reproductive organs that were fixed in Zenker's solution. A concern is that male rats were at the pubertal stage at the start of the study and were therefore past the age of maximum sensitivity to phthalate-induced testicular damage. However, mice were exposed during prenatal development (the most sensitive period for testicular toxicity) in a continuous breeding study described in the reproductive toxicity section.

There is adequate general toxicokinetic data for DnOP, consisting of absorption, distribution, metabolism, and excretion in rodents. While studies of toxicokinetics in humans have not been located, the DnOP toxicokinetic data in rodents is consistent with the large body of data on phthalates that includes data on rodents and primates. It is reasonable to assume that the DnOP rodent data is relevant to humans.

5.1.3 Developmental Toxicity

There are no data on the developmental toxicity of DnOP in humans. Two studies, where massive doses of DnOP were administered (4,890 and 9,780 mg/kg bw/day) to rats or mice, suggest a potential for adverse prenatal effect or effect during the perinatal period expressed as death, growth retardation, and/or malformations (*23, 24*). However, litter size and pup weight and mortality were unaffected in a continuous breeding study where mice were exposed to concentrations up to 7,500 mg/kg bw/day (*25, 26*). A primary metabolite, n-octanol, gave no sign of developmental toxicity at doses up to 1,300 mg/kg bw/day in rats (*27*). This dose caused severe intoxication and some deaths in the dams and prompts speculation whether the DnOP rat study that administered 4,890 mg/kg bw/day (*23*) led to severe maternal intoxication as well. The authors were silent on maternal effects. The limited study designs do not provide a basis for comparing consistency of response in the two species nor allow meaningful assessment of dose-response relationships. While the available studies do not allow determination of either LOAELs or NOAELs with any degree of confidence, the modest developmental toxicity response suggests that effects are likely to be associated with very high doses.

Utility of the Data to the CERHR Evaluation. The data set is inadequate for an evaluation of developmental toxicity. In one study, small numbers of rats (n=5/group) were exposed intraperitoneally, a route that is not relevant to human exposure, and there was no information on maternal toxicity. In a screening study of mice, only a single dose was administered and there was no internal examination of offspring or dams.

5.1.4 Reproductive Toxicity

There were no data located on the reproductive toxicity of DnOP in humans. There is a single multigeneration study on DnOP in mice which was negative at what can only be considered massive dietary doses, up to 7,500 mg/kg bw/day (*26*). This lack of effect is loosely corroborated by the dietary study in rats (*14*) which found no histologic effects on reproductive organs after sub-chronic exposure to concentrations as large as 350 or 403 mg/kg bw/day for males and females, respectively. Since there are no adverse reproductive effects in either study, no LOAEL can be estimated. The reproductive toxicity NOAEL in mice is 7,500 mg/kg bw/day and in rats is 350 mg/kg bw/day.

The Continuous Breeding design used is not a true multigeneration study because effective evaluation of the second generation is not performed. The 13-week feeding study of Poon et al. (*14*), in rats evaluated morphologic structure and weight where no biologically meaningful reproductive effects were seen.

The data are sufficient to say that DnOP causes no detectable reproductive toxicity in adult mice at doses up to ~7,500 mg/kg bw/day. The data in adult rats also find no reproductive toxicity at doses up to 403 mg/kg, but there are no data on functional measures of reproduction. The data are insufficient to conclude that DnOP does not cause reproductive toxicity in developing rats or mice. It can be reasonably speculated, based upon both *in vivo* and *in vitro* studies, that DnOP is certainly less potent in producing male reproductive effects than the shorter chain phthalate congeners.

Following exposure to a variety of phthalate monoesters over a range of doses, germ cell detachment was examined in *in vitro* co-cultures of Sertoli-germ cells isolated from pubertal rats. Results indicate that the n-octyl monoester is ~100-fold less potent than the 2-ethylhexyl monoester in producing this effect (*28*). These co-culture *in vitro* studies suggest that DnOP produces a similar effect to other phthalates in this model system, albeit at concentrations two orders of magnitude greater. There are no *in vivo* data to suggest effects on either germ cells or Sertoli cells due to DnOP exposure.

DnOP did not exhibit estrogenic activity in a variety of *in vitro* assays (*29*). It also did not induce a significant *in vivo* response in ovariectomized rats (*29*). The results suggest that adverse effects as a result of exposure to DnOP would not be due to estrogenic activities of this phthalate.

Utility of Data to the CERHR evaluation. Data are sufficient to indicate that oral DnOP exposures are not associated with detectable effects on reproduction at doses of up to 7,500 mg/kg bw/day in mice. Adequate numbers of mice (20 pairs/group) were exposed to multiple doses of DnOP for a sufficient duration. Feed was analyzed for DnOP levels. Reproductive function and sperm quality was assessed in the F_1 mice that were exposed during prenatal development; thus, mice exposed during the most sensitive age were evaluated. A concern with the study is that several postnatal maturation effects (found to be the most sensitive indicators of toxicity for other phthalates) were not evaluated. Other items of concern included no reporting of histopathological effects, examination of only the F_1 mice from the high-dose group , and a lack of necropsies at the lower dose levels.

5.2 Integrated Evaluation

There are no human data from which to judge the health effects of DnOP. Based on experimental literature, including toxicity studies in rats and mice with DnOP and other structurally related phthalates, there is a reasonable basis for assuming relevance of these data for judging potential hazard to humans.

There is no data indicating that DnOP is used in medical devices. Exposure to DnOP results from its presence as a 20% constituent of a commercial mixture of C6–10 phthalates. Humans would gain contact from household and consumer products. Absorption through skin from such contacts would be expected to be low. Absorption into the body would result from dietary sources. Presence in food might reflect migration from food packaging and a legacy of fate and transport of phthalates into the environment. Like other phthalates, DnOP is readily absorbed from the intestinal tract as a monoester, and is rapidly metabolized and excreted.

The experimental animal data are insufficient to permit a firm judgment about DnOP's potential to pose a developmental toxicity hazard to humans. Studies that suggest potential developmental effects were of inadequate design for confident interpretation and effects were observed only at very high doses. A study of n-octanol, a primary metabolite of DnOP, reported severe maternal intoxication without any effect on growth, viability, or development. It was noted that adequate data are available on DnOP to indicate adverse effects on liver at doses that are lower than doses suggestive of developmental toxicity. There are data to indicate that DnOP does not demonstrate estrogenic properties.

There are experimental data on the reproductive toxicity of DnOP. The data indicate no effects in adult mice fed high doses (7,500 mg/kg bw/day); the data in adult rats, while negative at dietary doses up to 350 mg/kg bw/day, did not assess a sufficient array of reproductive measures to be considered a complete evaluation. The data, while indicating a lack of effect, are insufficient to conclude with complete confidence that exposure by the oral route poses no hazard to adult reproduction. While there was a continuous breeding study that assessed the effects of exposure to DnOP during development on subsequent reproductive function later, the protocol did not completely assess two generations.

Summary

DNOP is present in 6,10-phthalate substances used as plasticizers in numerous consumer products. In the general public, exposure to DNOP is expected to occur primarily through food. Based on production volumes of 6,10-pthalate substances, the Expert Panel believes that general population exposure to DNOP will not exceed exposure estimates of 3–30 µg/kg bw/day, the estimates derived for DEHP. It is expected that DNOP is poorly absorbed through the skin; however, when ingested, a portion of DNOP is metabolized to a monoester, absorbed into the systemic circulation, further metabolized and rapidly excreted.

Most of the toxicological data were collected from studies in rodents. There are adequate oral studies to indicate that the major target organ for effects in rodents is the liver. A continuous breeding study in mice, with a limited assessment of the high-dose F_1 pups, produced no evidence of functional or structural reproductive toxicity at a dose up to 7,500 mg/kg bw/day. In a subchronic dietary exposure study, testicular lesions were not observed in rats exposed to up to 350 mg/kg bw/day. The data, while indicating a lack of effect, are insufficient to conclude with complete confidence that exposure by the oral route poses no hazard to adult human reproduction. The Expert Panel concluded that the data are insufficient for a confident assessment of DNOP-induced reproductive toxicity in humans.

Developmental effects were observed following exposure to a single high oral dose in mice or two high intraperitoneal doses in rats. The Expert Panel concluded that the study designs are inadequate for an assessment of DNOP-induced developmental toxicity in humans.

5.3 Critical Data Needs:

DnOP is a significant constituent (20%) of a C6–10 phthalate product that has major commercial production and use. The public may be better served if data needs for the evaluation of risks to human reproduction focus on the commercial mixture that contains DnOP rather than pure di-n-octyl phthalate. The scope and quantity of data would be similar to those identified in the evaluation of DnOP. Specifically:

1. Developmental toxicity studies in two species, using relevant doses and oral route of exposure, that span prenatal and postnatal periods of development and assess morphological and functional endpoints, including a focus on the reproductive axis.
2. A two-generation study in rats by the oral route of exposure.

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